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EFFECT OF GARLIC (*ALLIUM SATIVUM* L.) EXTRACT ON DEGREE OF HYDRATION, FRUCTOSE, SULPHUR AND PHOSPHORUS CONTENTS OF RAT EYELENS AND INTESTINAL ABSORPTION OF NUTRIENTS

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Influence of aqueous garlic extract on degree of hydration, fructose, sulphur and phosphorus contents of rat eyelens and intestinal absorption of nutrients were assessed. Inclusion of garlic extract in culture medium containing glucose and xylose inhibited the hydration of rat eyelens, whereas galactose evinced the reverse trend. Aqueous garlic extract in general decreased the concentration of fructose and phosphorus, whereas sulphur concentration increased when rat eyelenses, were incubated with galactose and xylose. Garlic extract inhibited intestinal absorption of glutamic acid, sucrose and glucose to different extents. The rate of absorption of glutamic acid was found to be considerably higher than that of glucose and sucrose.

KEY WORDS

Garlic, allium, medicinal value, degree of hydration, rat eyelens, nutrients absorption, drug equivalents.

Garlic (*Allium sativum* L) is an important and commercial bulbous crop that is popularly available both at immature and mature stages, but consumed to a limited extent due to reserved and unreserved preferences of the population. Garlic possesses higher nutritive value than other bulbous crops and rich source of carbohydrates, proteins and ascorbic acid (1). Critical appraisal of antioxidant and lipid oxidation of foods reviewed recently showed antioxidant capacity of 232 to garlic (2). Therapeutic properties of garlic have been recognized in processing of garlic capsules, tablets and other formulations related to human health (3). It has been also valued since long for its characteristic pungency, flavour and wide ranging medicinal properties due to accumulation of high concentration of different metabolites that constitute the basis for drug equivalents (3-5). Aqueous garlic extract contains the water soluble thiosulfinates and have strong antimicrobial activity (6, 7). Studies on phenols, structural carbohydrates and levels of peroxidase and acid phosphatase were also carried out during garlic bulb development (8). The effectiveness of pyruvate in protecting the light induced damage in isolated eyelens was also demonstrated (9). However, limited and scattered information exists on animal studies emphasizing intestinal nutrient utilization and on hydration of eyelens and its major metabolites as affected by garlic extract. The present investigation was undertaken to provide data on the above aspects that may further explore medicinal potential of garlic.

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MATERIALS AND METHODS

Garlic material : Mature garlic bulbs of 'G-1 Yamuna Safed' were procured from the Joint Director of National Horticulture Research and Development Foundation, Salru (Karnal). Aqueous garlic extract was prepared by crushing garlic cloves with five times of its weight of double distilled water and filtered through milipore filter (0.45 μm).

Experimental animals : Three weeks aged male albino weanling rats were obtained from the disease and germ free animal house of CCS Haryana Agricultural University, Hisar. Rats were fed *ad libitum* on nutritionally complete casein diet at 8% protein level containing salt mixture 3.5 per cent, vitamin mixture 2%, vitaminised groundnut oil 5 per cent, cellulose 5 per cent and corn starch to equal 100 per cent (10). The rats were housed individually

in aluminium metabolic cages and were kept in an air-conditioned room maintained at 22-24°C and having free access to water, food and air. After 38 days of experimental period, rats were kept on fasting overnight to create post absorptive state and were sacrificed by ether anaesthesia. Isolated eyelens and everted intestinal sacs of these rats were used in the present study.

Radio chemicals : D-glucose-^{14}C(U), specific activity 292 mCi/mmole, Sucrose-^{14}C(U), specific activity 64 mCi/mmole and L-glutamic acid-^{14}C(U), specific activity 146 mCi/mmole were purchased from Bhaba Atomic Research Centre, Trombay (Mumbai).

Degree of hydration of eyelens and metabolites assay : Isolated eyelenses were inoculated in glucose, galactose and xylose (50 mM) each in presence and absence of garlic extract (0.1 ml) using the method of Srivastava and Afaq (11). The weights of eyelenses were recorded after an interval of 1 hr up to 4 hr after gently removing the adhering liquid. After culturing eyelens for 4hr, they were washed with several changes of deionized water and inactivated in 2 ml of 1 N HCl and were disintegrated at 100°C for 20 min and the final volume was made to 5 ml with 1 N HCl. The phosphorus and sulphur contents were estimated by the method of Dickman and Bray (12) and Chesnin and Yien (13) respectively. The method of Roe (14) was employed for fructose estimation.

Preparation of intestinal sac : Intestinal sacs were prepared by the method of Mahmood *et al* (15). A 20-30 cm portion of the small intestine was removed immediately after anaesthesia by removing mesenteries carefully. The intestine was flushed with cold saline and everted using stainless steel rod carefully and slowly to avoid any injury to mucosal layer and the tissue was washed again in cold saline to remove any adhering material. Beginning with pyloric end, the tissue was dissected into 5-6 segments and end of each segment was tied with cotton thread and weighed. The sacs were filled individually with 0.5 ml of 5 mM glucose, sucrose and glutamic acid solution and tied the other end and weighed. Each sac was placed in 50 ml conical flask containing 5 ml of 5 mM respective solution and incubated at 37°C in a shaking water bath for 60 min. The intestinal sacs were removed and gently blotted to remove any adhering fluid and weighed. The fluid from the sac was drained by making a cut at one end, reweighed and dried in an oven at 110°C to constant weight.

Spectrophotometric assay : The contents of glucose and sucrose in the drained fluid were determined by anthrone reagent spectrophotometrically using Turner-350 spectrophotometer according to the method of Fong *et al* (16) and intestinal glutamic acid was assayed using ninhydrin by employing the method of Yemn and Cocking (17).

Intestinal absorption of ^{14}C labelled metabolites : Intestinal absorption of labelled compounds was determined following the method of Abedo *et al* (18). The filled sac containing respective metabolite solution was placed into a 25 ml conical flask having 5 ml of respective solution containing 5 mCi of the labelled compound. Incubated the flask in the same manner and decanted the intestinal sac in a separate petri dish as described earlier.

Measurement of radioactivity : The scintillation fluids were prepared according to Raghuramulu *et al* (19). To the decant solution (0.2 ml) in a glass scintillation vial, added 10 ml of scintillation fluid. Radioactivity of samples was assayed using the Liquid Scintillation counter Model Beckman LS180, USA with ^{14}C efficiency of 83.13 per cent and figure of merit of 99.15.

RESULTS AND DISCUSSION

The rat eyelens weight varied from 82.5 to 172.5 mg/100g body wt. After 1 hr of incubation, the degree of hydration increased in all the treatments. At different periods of incubation, hydration of eyelens incubated in 50 mM glucose and xylose in general increased throughout the incubation period and the presence of garlic extract in the incubation medium resulted in a significant inhibition in the degree of hydration (Table 1). Conversely, hydration of eyelens in 50 mM galactose increased upto 1 hr and thereafter declined a little. Addition of garlic extract in this treatment increased the hydration of eyelens during 4 hr of culturing as compared to 0 hr. Culturing of eyelens in glutamic acid alone and in combination with garlic extract resulted in a decrease in the degree of hydration after 1, 2, 3 and 4 hr in comparisoin to 0 hr. The results suggest that garlic extract possesses this property and the inhibitory effect is probably due to its sulphur compounds which are good acceptors of hydrogen and the corresponding activity may be due to their reaction with thiol group substances and NADPH.

Similar views were earlier expressed by Adamu *et al* (20). The present results are akin to the findings of Srivastava and Afaq (11) that garlic extract inhibits the increase in water content in rat eyelens cultured in a medium containing glucose and xylose but differs in response to galactose. Gupta *et al* (9) indicated that pyruvate has anticataract potential in controlling galactose induced cataract in isolated rat eyelens and garlic is a good source of pyruvate (8, 21-23). Determination of sulphur, fructose and phosphorus contents in rat eyelens after 4 hr of incubation showed that sulphur concentration in eyelens increased in garlic extract treatments as compared to controls when eyelenses were cultured in medium containing galactose and xylose only (Table 2). This may be due to transport of sulphur compounds contributed by garlic extract from the medium surrounding the eyelens. The inclusion of garlic extract decreased phosphorus in eyelenses when incubated with glucose and xylose but it remained unaltered in galactose treatment. Fructose concentration declined in all the garlic treatments. The decline in fructose and phosphorus contents in almost all the cases may be due to leaching of nutrients in the surrounding medium because of osmotic effect and altered eyelens metabolism (9).

Monitoring of nutrients absorption namely glucose, sucrose and glutamic acid by everted intestinal sac of rat by spectrophotometeric assay (Table 3) inferred that glutamic acid (1416.45 mg/h/g dry wt) was absorbed at a higher rate in comparison to glucose (752.30 mg/hr/g dry wt) and sucrose (744.96 mg/hrg dry wt). Scintillation measurement of ^{14}C labelled intestinal metabolites also showed that glutamic acid (908.81×10^4 CPM/hr/g dry wt) was absorbed to the greater extent than glucose (275.97×10^4 CPM /hr/g dry wt). Inclusion of garlic extract in the medium inhibited the absorption of all the metabolites. The probable reason for variation in nutrients absorption could be the size and structure of molecule that resulted in conditioning of surface area of the mucosal epithelial cells of the intestinal sacs and their corresponding receptors and enzymes implicated in absorption (24). Singh *et al* (25) and Deka *et al* (26) also viewed the differential pattern of nutrients by these techniques and reported good agreement between two assays. Sugar and amino acids are absorbed at different rates and absorption is mediated by carriers system and active transport that is regulated by Na^+-K^+-ATPase (27). Immature garlic bulbs are good source of Li (28) which is transported across cell membranes by Na+-K+ pump and exerts protective effect on the incidence of atherosclerotic heart diseases and interfere with vasopresin action (29). Blood sugar lowering effects of garlic were reported by Brahmachri and Agusti (30), Mathew and Augusti (31) and Jain *et al* (32) and in garlic it is mainly ascribed to allicin and disulphides containing compounds.

The present study may be helpful in further understanding the role of garlic extract in eyelens metabolism and intestinal absorption of nutrients.

Table 1

Influence of garlic extract on hydration of rat eyelens

Nutrients (amino acid/sugar, 50mM)	Eyelens weight (mg/100 g body wt)	Degree of hydration			
		1 hr	2 hr	3 hr	4 hr
Rat eyelens incubated in medium + glutamic acid	118.10	108.44	107.79	106.82	106.49
Rat eyelens incubated in medium + glutamic acid + garlic extract	116.50	113.11	112.07	113.77	112.13
Rat eyelens incubated in medium + galactose	99.32	106.47	105.76	104.47	104.09
Rat eyelens incubated in medium + galactose + garlic extract	100.34	107.66	108.09	107.23	105.11
Rat eyelens incubated in medium + xylose	82.50	119.76	102.24	132.73	136.97
Rat eyelens incubated in medium + xylose + garlic extract	172.50	114.78	113.04	116.52	116.20
Rat eyelens incubated in medium + glucose	115.03	109.78	111.11	112.89	112.89
Rat eyelens incubated in medium + glucose + garlic extract	118.61	105.17	109.48	109.05	104.74

Table 2

Effect of garlic extract on sulfur, fructose and phosphorus contents of rat eyelens

Treatments (Nutrient sugar, 50 mM)	Sulphur (μg/eyelens)	Fructose (μg/eyelens)	Phosphorus (μg/eyelens)
Rat eyelens incubated in medium + galactose	51.0	50.0	23.0
Rat eyelens incubated in medium + galactose + garlic extract	122.0	42.0	23.0
Rat eyelens incubated in medium + glucose	126.0	46.0	29.0
Rat eyelens incubated in medium + glucose + garlic extract	46.0	42.0	8.0
Rat eyelens incubated in medium + xylose	84.0	63.0	25.0
Rat eyelens incubated in medium + xylose + garlic extract	122.0	59.0	17.0

Table 3

Effect of garlic extrac on *in vitro* absorption of nutrients by everted intestinal sacs of rats

Nutrients	Absorption by spectrophotometer assay (mg substrate absorbed/ hr/g dry wt of sac)	Absorption by scintillation assay (CPM/hr/g dry wt of sac)
Glucose	752.30±1.12	$275.97\times10^4\pm0.435$
Glucose + Garlic extract	749.33±1.20	$273.71\times10^4\pm3.041$
Sucrose	744.96±1.72	$217.21\times10^4\pm0.466$
Sucrose + Garlic extract	738.77±5.88	$216.41\times10^4\pm4.153$
Glutamic acid	1416.45±7.62	$908.81\times10^4\pm0.868$
Glutamic acid + Garlic extract	1375.48±20.91	$892.58\times10^4\pm0.199$

Each value is an average of two estimations

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